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## FINAL TECHNICAL REPORT

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Principal Investigator: Benjamin H. Ershoff, Ph.D.

Co-investigators: G.S. Bajwa, D.V.M., Ph.D.,  
L.A. Bavetta, Ph.D., and  
M. Shapiro, D.D.S.

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9331 Venice Blvd.  
Culver City, California

Subject of the Report: Lesions in the oral tissues induced  
by exposure to multiple sublethal doses of  
total body x-irradiation in the mouse and the  
effects of dietary supplements of bioflavonoids  
thereon.

Contract Number: DA-49-193-MD-2210

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## A B S T R A C T

Preparing Institution: Western Biological Laboratories  
9331 Venice Blvd.  
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and M. Shapiro, D.D.S.

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The effects of multiple sublethal doses of total body x-irradiation were determined on the oral tissues of mice fed a highly purified basal ration and the basal ration plus supplements of bioflavonoids of diverse structure and physico-chemical properties. Similar lesions were observed in all x-irradiated mice with no significant differences between the various dietary groups. The lesions observed include rarefaction, collagenolysis, pyknosis, canalization with capillaries, and inclusions of squamous epithelium and osteoclasts of the periodontium; focal resorption of the acellular cementum; osteoclastic inclusions in the dentin; acute gingivitis and interdental pyorrhea; decreased trabeculation and bone marrow of the alveolar bone; and neoplastic changes of the lacrimal gland, submaxillary salivary gland and squamous epithelium. Lesions comparable to the above were not observed in non-irradiated mice in the various dietary groups.

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Previous findings from this laboratory (1) indicate that mice raised from weaning on a highly purified diet and exposed to multiple sublethal doses of total body x-irradiation subsequently developed marked lesions in the oral tissues. Periodontal lesions following exposure to multiple sublethal doses of x-irradiation have also been reported by Fedorov and Prokhonchukov (2) in the rat. Since certain bioflavonoids (naringin, hesperidin methyl chalcone and rutin) at levels of 2% or 4% of the diet increased the survival time of mice exposed to multiple sublethal doses of total body x-irradiation over that of similarly treated mice fed a comparable diet without these supplements whereas other bioflavonoids (hesperidin and lemon bioflavonoid complex) when fed at similar levels did not have this effect (3), experiments were undertaken to determine the effects of bioflavonoids of diverse structure and physico-chemical properties on the incidence and severity of periodontal lesions induced by multiple sublethal doses of total body x-irradiation in the mouse.

**Procedure.** The basal ration employed in the present experiment consisted of cerelose, 59%; casein<sup>1</sup>, 24%; cottonseed oil, 10%; salt mixture<sup>2</sup>, 5%; cellulose<sup>3</sup>, 2%; and the following vitamins per kg of diet: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; Vitamin B<sub>12</sub>, 150 µg; 2-methyl-1,4-naphthoquinone, 5 mg; choline chloride, 2 g; Vitamin A, 5000 U.S.P. units; Vitamin D<sub>2</sub>, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. The vitamins were added in place of an equal amount of cerelose.

Male mice of the Webster strain were selected at 11 to 14 g in body weight and were divided into comparable groups consisting of 40 animals per group. These were placed in metal cages with raised screen bottoms (5 animals per cage) and were fed either the basal ration or the basal ration plus the supplements listed below:

- |                                   |                                    |
|-----------------------------------|------------------------------------|
| (a) 1% hesperidin                 | (h) 2% naringin                    |
| (b) 2% hesperidin                 | (i) 1% naringenin                  |
| (c) 1% hesperidin methyl chalcone | (j) 2% naringenin                  |
| (d) 2% hesperidin methyl chalcone | (k) 2% lemon bioflavonoid complex  |
| (e) 1% hesperetin                 | (l) 2% orange bioflavonoid complex |
| (f) 2% hesperetin                 | (m) 2% rutin                       |
| (g) 1% naringin                   |                                    |

The supplements were added to the basal ration in place of an equal amount of cerelose. Food and water were provided ad libitum. The animals were fed daily and all food not consumed 24 hours after feeding was discarded. After 6 weeks of feeding 10 of the mice in each dietary group were selected at random to serve as non-irradiated controls. The remaining mice received an exposure of 200 r total body x-irradiation which was repeated once weekly until a total dose of 1200 r (6 exposures) had been administered.

<sup>1</sup>Vitamin-free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

<sup>2</sup>Wesson Modification of Osborne-Mendel Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

<sup>3</sup>Solka Floc, Brown and Co., Boston, Mass.

The animals to be irradiated were placed in a wooden box divided into 60 equal compartments,  $1\frac{1}{4}$  inches wide, 3 inches long, and  $1\frac{1}{2}$  inches deep. The partitions and top were made of 1/8-inch cellulose acetate sheeting. The top and bottom of each compartment were perforated with holes for purposes of ventilation. The container was rotated slowly on an electrically driven turntable to insure equivalent exposures. The following radiation factors were employed: GE Model Maximar 250; 250 kv; 15 mg; 0.5 mm. Cu and 1 mm. Al filters plus a Cu parabolic filter<sup>4</sup>; HVL, 2.15 mm. Cu; target distance to top of box, 82 cm.; and dose rate, 15.6 r/min (measured in air, at top of box). Surviving mice were sacrificed 120 days after the first x-irradiation; the heads were fixed in 10% neutral formalin, decalcified in 10% nitric acid in 10% formalin and washed with saturated lithium carbonate solution. They were then dehydrated and infiltrated in the routine manner; and paraffin sections prepared, sectioned at 7  $\mu$  thickness, and stained with hematoxylin and eosin.

#### HISTOPATHOLOGICAL FINDINGS

Four animals were randomly selected from each group of mice for histologic examination of the head. The non-irradiated mice showed only occasional inflammatory foci; whereas, lesions occurred in all x-irradiated mice without any significant differences between those fed the basal ration and those fed the test supplements. Pathological changes in x-irradiated mice appeared in a regular pattern and were as follows:

**PERIODONTIUM:** Periodontal lesions occurred in all mice and were often very striking. These were marked by increased width, rarefaction (cellular sparsity) (Fig. 1A), canalization with capillaries (Figs. 1B, C & E), pyknosis (Fig. 1B), collagenolysis (Fig. 1B), and inclusions of bony spicules, squamous epithelium and/or osteoclasts (Fig. 1C). The above alterations were present as follows:

<u>Alteration</u>	<u>Per Cent</u>
Increased width	73.2
Rarefaction (cellular sparsity)	100.0
Canalization with capillaries	100.0
Pyknosis	100.0
Collagenolysis	100.0
Bony spicules	91.4
Squamous epithelial inclusions	72.2
Osteoclastic inclusions	78.8

Profuse myelogenous neoplastic invasion was seen in two animals. Generally, the lesions were most severe adjacent to the 1st molar.

**CEMENTUM:** Focal resorption, especially of the acellular cementum, was a common finding, and it was apparently due to invasion by osteoclasts originating from the adjacent alveolar bone. These areas were seen as indentations, filled mostly with periodontal cells

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<sup>4</sup>A nonuniform filter which produces a flat isodose surface of x-ray intensity constructed by the method of Greenfield and Hand (4). The center of the filter had a thickness of 1.7 mm Cu; the edge, 0.5 mm Cu.

and occasionally with osteoclasts (Fig. 1C). Osteoclastic and periodontal cell inclusions occurred in 29.6 and 77.5% of the teeth, respectively. The inclusions were seen exclusively in the 2nd and 3rd molars, and were conspicuously absent in the 1st molar and incisors.

**DENTAL PULP:** Lesions of the pulp were uncommon but when present were confined principally to disorientation of the odontoblasts resulting in ectopic dentification in 28.2% of the teeth (Fig. 1D). Caseation necrosis of the entire pulp was noted in one molar.

**DENTIN:** Of various tissues of the tooth, dentin showed the least alterations. The major lesions consisted of osteoclastic invasion following complete resorption of the cementum, and these were seen as pockets enclosing osteoclasts in 16.9% of the molars (Fig. 1E). Severe dentinolysis occurred in one molar.

**GINGIVAE:** Mild to severe, acute gingivitis was commonly seen as marked by heterophilic infiltration and variable superficial necrosis of the gingival squamous epithelium (Fig. 1F). Various lesions occurred as follows:

<u>Lesion</u>	<u>Per Cent</u>
Heterophilic infiltration	70.0
Intendental pyorrhea	36.6
Pyorrhea of the lower incisor	11.2
Neoplastic invasion	2.8

Necrosis of the periodontal membrane usually accompanied pyorrhea of the lower incisor.

**ALVEOLAR BONE:** The lesions of the alveolar bone were almost as common as those of the periodontium, and were characterized by depressed alveolar crest, decreased trabeculation (Fig. 1A), increased basal bone width (Fig. 1A), increased resorptive and osteoclastic activity (Fig. 1C), and decreased bone marrow. These lesions were present as follows:

<u>Lesion</u>	<u>Per Cent</u>
Depressed alveolar crest	57.7
Decreased trabeculation	95.8
Increased basal bone width	93.0
Increased resorptive and osteoclastic activity	78.9
Decreased bone marrow	93.0

Extensive myelogenous neoplasia of the bone marrow was observed in two mice.

**LACRIMAL GLAND:** The changes in the lacrimal gland were principally neoplastic in nature. Precancerous and cancerous lesions were observed in 26.7% of the animals (Fig. 2A). The affected glands were usually cystic, and showed focal to diffuse stromal proliferation.

**SUBMAXILLARY SALIVARY GLAND:** The gland exhibited changes similar in nature to those of the lacrimal gland, but less frequently. Stromal proliferation and precancerous changes were present in 17% of the mice.

**BRAIN:** The brain was commonly affected as shown by capillary engorgement in 86.6%, and glial satellitosis and degeneration of the cerebral neurons in 92% of the mice. Neoplastic invasion of the meninges and gasserian ganglion was seen in one animal (Fig. 2B).

**THYROID:** Seven of the 10 glands examined showed marked peripheral vacuolation of the colloid material (Fig. 2C).

**HAIR FOLLICLE:** Folliculitis and adjacent cellulitis occurred in 45% of the upper lips.

**MANDIBULAR LYMPH NODE:** Eight of the 8 lymph nodes examined showed lymphocytic depletion, and sinusoidal macrophagic proliferation (Fig. 2D). One animal revealed profuse myelogenous neoplastic invasion causing severe obliteration of the normal architect of the lymph node.

**SQUAMOUS EPITHELIUM:** Precancerous changes were present in 13% of the mice (Fig. 2E).

**GENERALIZED NEOPLASIA:** Neoplastic (myelogenous) invasion of various tissues of the head was striking in two mice (Fig. 2A & F).

Generally, the aforementioned lesions were closely similar in extent, distribution and nature from animal to animal. The differences among the animals were merely quantitative.

Summary. The effects of multiple sublethal doses of total body x-irradiation were determined on the oral tissues of mice fed a highly purified basal ration and the basal ration plus supplements of bioflavonoids of diverse structure and physico-chemical properties. Similar lesions were observed in all x-irradiated mice with no significant differences between the various dietary groups. The lesions observed include rarefaction, collagenolysis, pyknosis, canalization with capillaries, and inclusions of squamous epithelium and osteoclasts of the periodontium; focal resorption of the acellular cementum; osteoclastic inclusions in the dentin; acute gingivitis and interdental pyorrhea; decreased trabeculation and bone marrow of the alveolar bone; and neoplastic changes of the lacrimal gland, submaxillary salivary gland and squamous epithelium. Lesions comparable to the above were not observed in non-irradiated mice in the various dietary groups.



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3. Ershoff, B.H., and Steers, Jr., C.W. Bioflavonoids and survival time of mice exposed to multiple sublethal doses of x-irradiation, Proc. Soc. Exp. Biol. and Med., 105: 283, 1960.
4. Greenfield, M.A., and Hand, K. Non-uniform filter to produce a flat isodose surface of x-ray intensity, Am. J. Roentgenol. Radium Therapy, 68: 960, 1952.



Fig. 1A



Fig. 1B



Fig. 1C



Fig. 1D



Fig. 1E

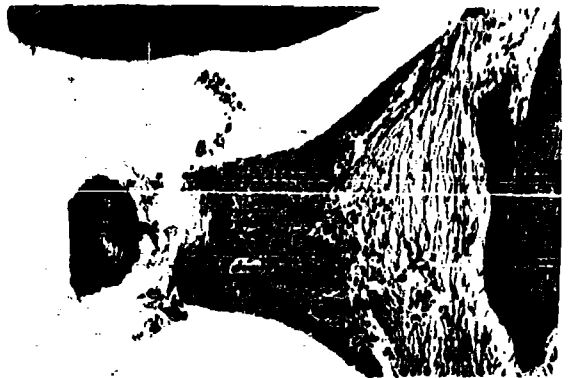


Fig. 1F

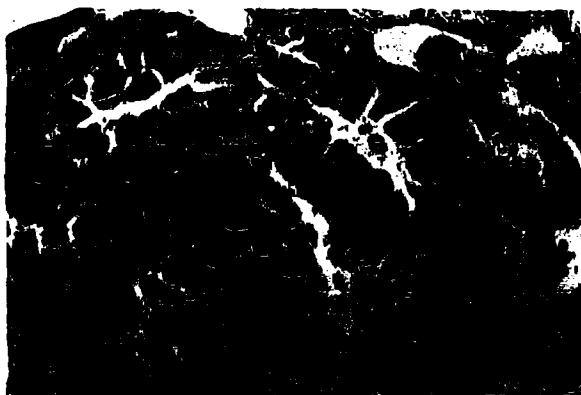


Fig. 2A



Fig. 2B



Fig. 2C

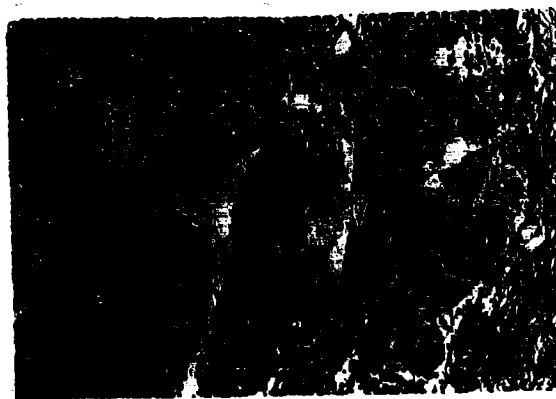


Fig. 2D

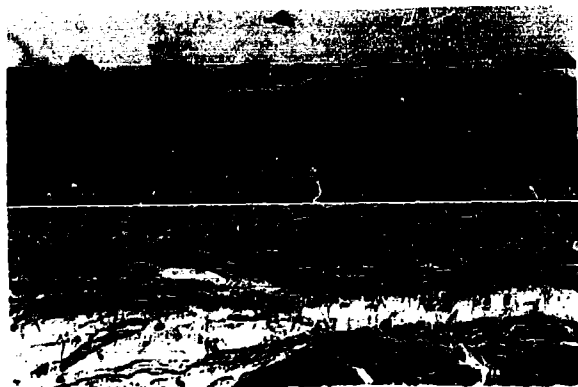


Fig. 2E



Fig. 2F

## LEGEND TO FIGURES

Fig. 1A. Typical rarefaction of the periodontium of 1st molar showing cellular sparsity; and increased width of the basal alveolar bone. H & E stain x160.

Fig. 1B. Collagenolysis, pyknosis, and canalization with capillaries of the periodontium of 1st molar. H & E stain x240.

Fig. 1C. Second molar showing squamous epithelial, osteoclastic and osseous inclusions in the periodontium; focal osteoclastic resorption and periodontal cell inclusion of the acellular cementum; and increased resorptive and osteoclastic activity of the alveolar bone. H & E stain x240.

Fig. 1D. Ectopic dentification in the pulp of a second molar. H & E stain x160.

Fig. 1E. Osteoclastic inclusion in the dentin of a second molar. H & E stain x240.

Fig. 1F. Interdental pyorrhea seen as heterophilic infiltration and superficial necrosis of an interdental gingivae crest. H & E stain x160.

Fig. 2A. Papillary adenomatosis of a lacrimal gland. H & E stain x160.

Fig. 2B. Neoplastic (myelogenous) invasion of the meninges and gasserian ganglion. H & E stain x160.

Fig. 2C. Peripheral vacuolation of the colloid material of a thyroid. H & E stain x240.

Fig. 2D. Profuse macrophagic proliferation in the sinusoids of a mandibular lymph node. H & E stain x160.

Fig. 2E. Precancerous lesion of squamous epithelium of the mucosal surface of an upper lip. H & E stain x240.

Fig. 2F. Neoplastic invasion (dark staining cells) of a lacrimal gland. H & E stain x160.

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